Pulmonary Angiotensin-converting Enzyme (ACE) Binding and Inhibition in Humans

A Positron Emission Tomography Study

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Angiotensin-converting enzyme (ACE) inhibition attenuates pulmonary hypertension and delays the development of pulmonary vascular remodeling in animal models. Thus, ACE inhibition might be a useful treatment for primary pulmonary hypertension (PPH). To determine the dose of ACE inhibitor required to specifically block pulmonary ACE in humans, we measured the combined forward rate constant (CFRC) for [^{18}F]-fluorocaptopril, which is proportional to the mass of ACE in the lung, using positron emission tomography (PET). In five normal subjects, CFRC was measured twice, 1 wk apart, to assess measurement reproducibility. The CFRC was 0.151 ± 0.067 for the first measurement and 0.140 ± 0.060 for the second measurement (p = not significant [NS]). In five normals, CFRC decreased on average 84%, from 0.177 ± 0.053/s to 0.028 ± 0.017/s (p < 0.05), after 1 wk ingestion of 5 mg enalapril orally once a day (the scans were performed 24 h after the last medication). Similarly, in five patients with PPH, CFRC decreased on average 76%, from 0.052 ± 0.020/s to 0.012 ± 0.003 (p < 0.01), after 1 wk enalapril, despite much lower baseline values. We conclude that the total mass of pulmonary ACE appears to be significantly reduced in PPH and that only low doses of ACE inhibitors may be needed to block the effects of ACE on vascular remodeling in PPH.

Vascular remodeling, which is characterized by pulmonary artery medial hypertrophy, neointimal formation, and thrombosis *in situ*, is an important component of the pathogenesis of primary pulmonary hypertension (PPH) (1–4). As the lesions of vascular remodeling develop, they contribute to the high vascular impedance that ultimately results in right ventricular failure and death.

The expression of factors that are likely to promote remodeling, such as transforming growth factor-β, angiotensin-converting enzyme (ACE), and endothelin-1, is increased at the sites of active remodeling (5–9). Recently, several studies provide evidence that ACE expression is altered in both patients with PPH and in animal models of pulmonary hypertension (9–11). Thus, ACE inhibitors, which reduce angiotensin II production (with its growth-promoting effects) and bradykinin degradation (thereby promoting its antiproliferative effects), may favorably alter pathologic remodeling in response to tissue injury (12). Such effects may be responsible for the clinical benefits associated with the use of ACE inhibitors in a number of different conditions, including myocardial infarction, hypertension, and renovascular disease (13), providing a rationale for their use as therapy in PPH.

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Even so, any possibility of evaluating the benefit of ACE inhibitors in PPH must be undertaken with caution, because these drugs can cause systemic hypotension and patients with incipient or frank right ventricular failure may be especially sensitive to such effects. Ideally, it would be useful to be able to determine the least amount of ACE inhibitor necessary to specifically block pulmonary ACE. We decided that this goal could be achieved with a newly developed technique based on imaging with positron emission tomography (PET) and fluorine-18 labeled fluorocaptopril, [^{18}F]FCAP, which, like captopril itself, binds to ACE with high specificity. The results suggest that only modest and well tolerated doses of ACE inhibitors may be required to specifically block lung ACE, making it feasible to design a safe study of the impact of ACE inhibitors in PPH.

METHODS

Theory

We have previously described a new PET imaging procedure to evaluate lung ACE kinetics in intact animals (14, 15). The method is based on sequential imaging after the intravenous administration of [^{18}F]FCAP. Tissue time–activity curves are obtained from the sequential images and analyzed with a 3-compartment mathematical model. Radioactivity from [^{18}F]FCAP in blood perfusing lung tissue regions is represented in two of these compartments, where activity in the first compartment is the "input function," i.e., activity entering the tissue regions. Compartment 2 represents activity in the remaining vascular space in which [^{18}F]FCAP can bind to ACE. Compartment 3 represents activity actually bound to ACE.

As originally reported, the method is used to estimate the maximal binding concentration of [^{18}F]FCAP to ACE in the lung (B_{max}). In theory, then, one might hope to simply measure B_{max} before and after the administration of an oral ACE inhibitor to determine the dose of ACE inhibitor required to block lung ACE (i.e., to decrease B_{max}). However, estimates of B_{max} require a separate injection of unlabeled captopril to displace the radioactive [^{18}F]FCAP that is bound to ACE. Such displacement reactions would not be detectable in the presence of significant binding by an unlabeled ACE inhibitor (administered therapeutically). Thus, in the present study, we determined the estimated rate for binding to ACE (the combined forward rate constant [CFRC]), which is the product of the association rate constant (k_a) and the concentration of ACE binding sites (B_{max}). CFRC can be calculated from a single trace injection of radiolabeled [^{18}F]FCAP. With the assumption that the association rate constant k_a for [^{18}F]FCAP would not be altered by occupancy of some portion of total ACE by an ACE inhibitor, a reduction in the measured CFRC is equivalent to a reduction in B_{max}, representing in this case occupancy by the unlabeled ACE inhibitor.

Subjects

Thirteen normal volunteers (7 male, 6 female), ages ranging between 23 and 70 yr, were studied in two different protocols. In the first protocol, CFRC was determined in 10 subjects, five of whom where studied twice, 1 wk apart, without any intervention, to assess the reproducibility of the technique in human subjects. In the second protocol, CFRC was measured in five subjects (two of whom participated in the first protocol) before treatment, and then again 24 h after the final dose of a 1-wk daily administration of enalapril (an oral ACE inhibi-

tor) (5 mg once a day) to determine the extent of blocking (i.e., drug occupancy) with this low dose of drug.

CFRC was also measured in six patients with PPH (Table 1). All patients had a documented mean pulmonary artery pressure greater than 25 mm Hg at rest and had been previously evaluated for secondary causes of pulmonary hypertension.

In five of the patients with PPH, CFRC was measured before treatment, and then again 24 h after completion of 1 wk of treatment with 5 mg enalapril once a day, as in the normal subject group.

Subjects participated in this study after giving written and informed consent to the relevant protocols which were approved by the Radioactive Drug Research Committee and Human Studies Committee at Washington University School of Medicine.

PET Scanning

PET scans were performed using a Siemens/CTI ECAT EXAC HR plus 962 scanner, a 63-plane positron camera with an axial sampling of 2.43 mm, isotropic resolution 4.6 mm, and a 15 × 56 cm field of view

TABLE 1

DEMOGRAPHIC INFORMATION ON PATIENTS WITH PPH

Patient No.	Gender	Age	Ppa	Years Since Diagnosis	PGI_2 or CCB	NYHA Class
1	M	38	90/60	2	PGO_2	III/IV
2	F	40	74/36	2	—	II/III
3	F	49	74/29	3	both	II/III
4	M	75	35*	1.5	both	III/IV
5	F	43	68†	4	both	II
6	F	44	85†	2.5	—	III/IV

Definition of abbreviations: CCB = calcium channel blocking drug; NYHA = New York Heart Association; PGI_2 = prostacyclin; Ppa = pulmonary artery pressure; PPH = primary pulmonary hypertension.

* Mean pulmonary artery pressure only available.

† Systolic pulmonary artery pressure estimated by cardiac echocardiography.

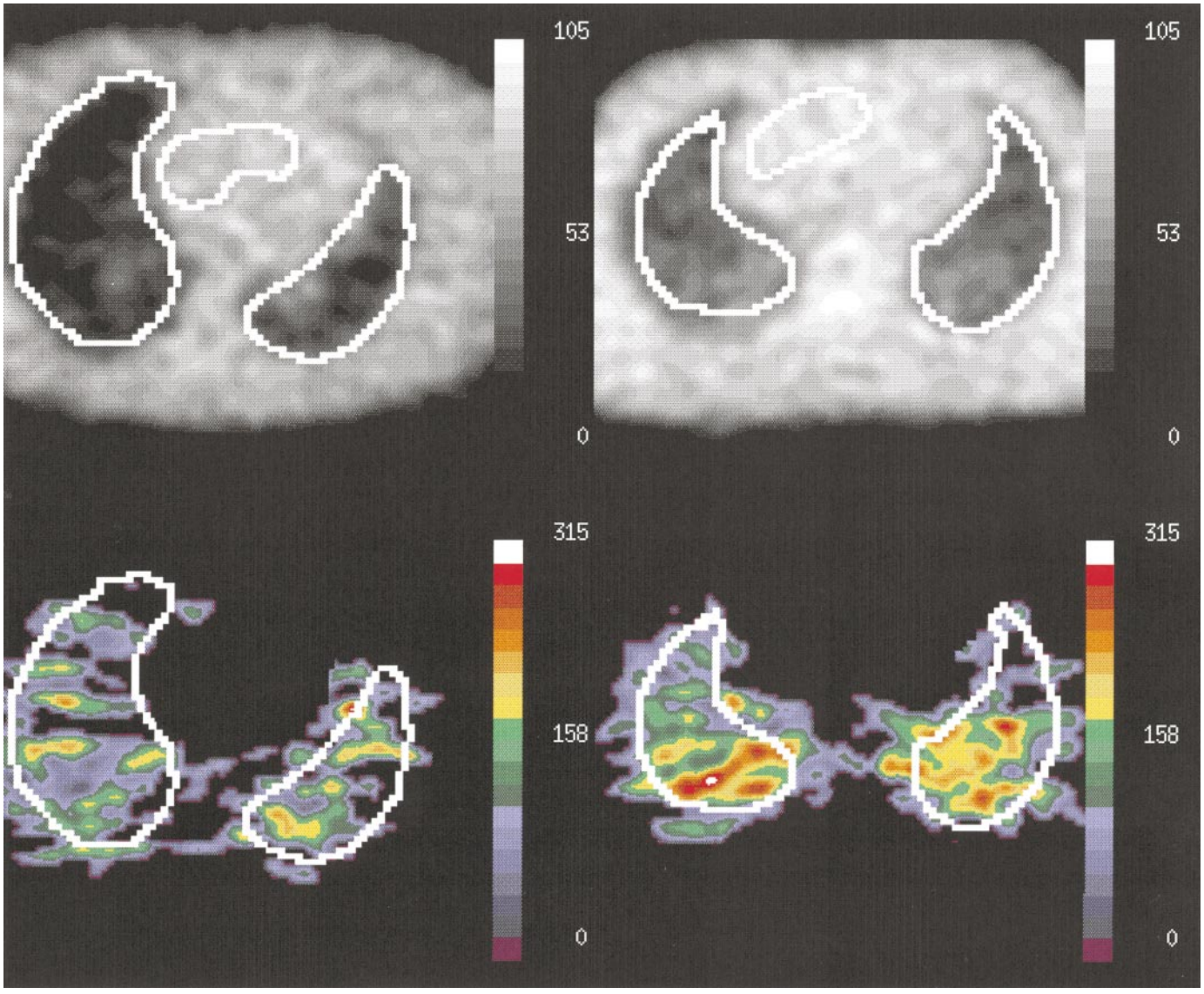

Figure 1. Representative PET images from one patient with PPH (*left side*) and one normal subject (*right side*). *Upper left*: a transmission scan, used to correct emission images for the effects of photon attenuation, and to show method of placing ROIs for analysis. (The central region is placed over the right ventricle, as determined from Vb and blood flow images; not shown (*see* METHODS). The image gray scale is proportional to tissue density. *Lower left*: a 30-s [^{18}F]FCAP image taken at the end of the imaging protocol (*see* METHODS). The false color scale is in units of tissue activity (counts/s/100 ml lung). Comparable images for the normal subject are shown on the right side of the figure. Note the reduced amount of [^{11}F]FCAP activity in the patient with PPH, consistent with reduced lung uptake of the tracer.

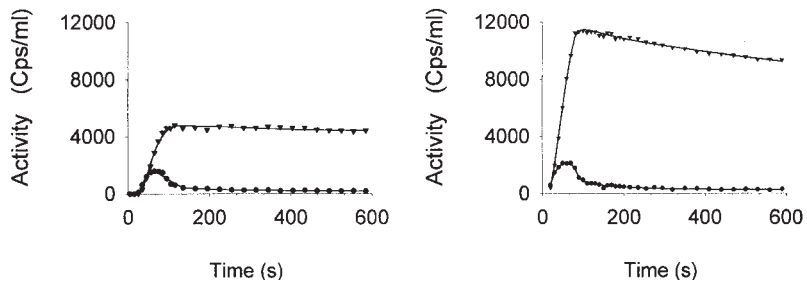

Figure 2. Time–activity data after the administration of [^{18}F]FCAP in one patient with PPH (*left side*; same patient as in Figure 1) and one normal subject (*right side*, same subject as in Figure 1). *Triangles* represent data from a single lung ROI; *circles* represent data in the Vb within the same region (*see* METHODS). Data for the PPH patient have been normalized for dose for purposes of this figure only, so that relative differences with the normal subject are readily apparent. When data from all image regions were combined, the CFRC for the patient with PPH was calculated to be 0.06 s^{-1}, and 0.12 s^{-1} for the normal subject. Both values are near the mean group values respectively (*see* RESULTS).

(FOV). To improve signal-to-noise of the activity measurements, we reduced the data to 10 slices (for each time frame) by combining six original planes to form a single transverse slice with an axial sampling of 15 mm.

PET scanning consisted of transmission, oxygen-15-labeled carbon monoxide ($C^{15}O$) emission, oxygen-15-labeled water ($H_2{}^{15}O$) emission, and [^{18}F]FCAP dynamic emission scans. Methods associated with using these radiopharmaceuticals to measure regional pulmonary blood volume (Vb), pulmonary blood flow, and ACE kinetics have been summarized in Reference 16.

The patients lay in the supine position. A venous cannula was inserted into a forearm vein for tracer infusion. Arterial blood pressure, electrocardiogram (ECG), and O_2 saturation were monitored throughout the study.

A 15-min transmission scan was obtained first. This scan was used to correct subsequent emission data for attenuation effects and also to provide images of lung and chest density which were used to define regions of interest (ROI) on the lung images.

Next, a 5-min scan was performed 2 min after a single deep breath of $C^{15}O$ (approximately 40 mCi). This scan was used to calculate regional pulmonary Vb (ml/ml lung).

Next, approximately 50 mCi $H_2{}^{15}O$ was injected in two fractions into a forearm vein. Approximately 25 mCi of activity were first injected by hand over a 10-s period. Activity data were collected at the start of the injection for 60 s. The distribution of this activity in the lungs is proportional to regional pulmonary blood flow (PBF). At the end of this period, the second fraction of activity (approximately 15 mCi after decay) was injected as a bolus. Then 4 min after the initial injection, a second PET data collection was started to determine the apparent regional partition coefficient for the tracer (necessary for the calculation of PBF).

Finally, a series of images was obtained after the intravenous injection of [^{18}F]FCAP. The 4-cis-[^{18}F]flurocaptopril was synthesized by the displacement reaction of the corresponding triflate precursor with K^{18}F/Kryptofix 222 in acetonitrile followed by hydrolysis and purification as described previously (17). Between 2 and 3.75 mCi of [^{18}F]FCAP (in 20 ml of normal saline) was infused through the forearm vein cannula by an electronic pump over 60 s followed immediately by 20 ml of normal saline flush during another 60-s period. Fifty-eight dynamic time frames (twelve 10-s + forty-six 30-s frames) were obtained, starting at the time of [^{18}F]FCAP injection, over 25 min.

Image Analysis

ROIs for the lung were drawn on the transmission images (Figure 1). Each ROI included all lung image elements on each side of the thorax for that image (two "hemislices"). The hila, chest wall, and diaphragm were excluded from such regions. Typically, ROIs were drawn on six of 10 planes.

Images for PBF and Vb were processed as previously described (18). The mean PBF for each hemislice region was expressed in units of ml blood/min/100 ml lung, and as ml blood/min/kg/100 ml lung to correct for body weight. Vb values were expressed as ml blood/ml lung.

To generate pulmonary tissue time–activity curves for the calculation of CFRC, the same ROIs were projected onto the dynamic [^{18}F] FCAP images. The mean tracer activity in serial lung planes (craniocaudal) was calculated and plotted against time (Figure 2).

Implementation of the mathematical model used to estimate ACE kinetic parameters requires a measurement of blood activity entering each ROI ("input function"), in addition to the total activity within the ROI. Instead of sampling and counting pulmonary artery blood activity directly, we estimated the input function directly from the PET images themselves, as follows. First, an ROI for the right ventricle was drawn on Vb images (Figure 1). Second, pixel values within this ROI for the right ventricle were obtained for each of the dynamic [^{18}F]FCAP images, allowing us to obtain time–activity data from the right ventricle. These data were then corrected for partial volume averaging (owing to the small size of the right ventricle relative to the

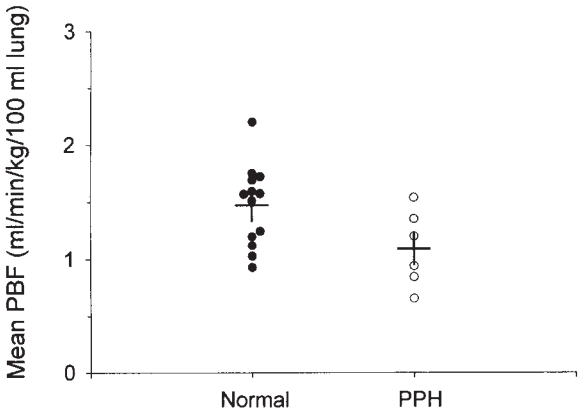

Figure 3. Mean ("+" sign) and individual (*symbols*) PBF measurements in 13 normal subjects and six patients with PPH, as measured with PET imaging. The mean values are significantly different ($p < 0.05$).

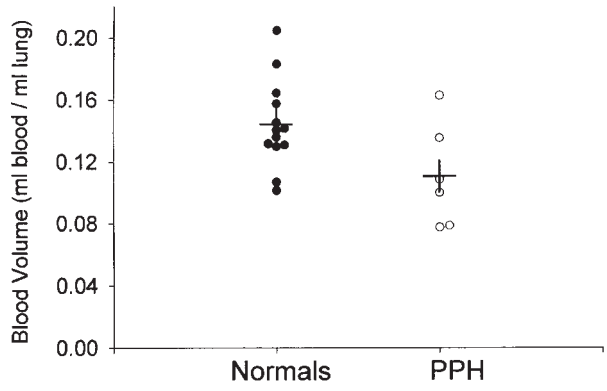

Figure 4. Mean ("+" sign) and individual (*symbols*) regional pulmonary V_b measurements in 13 normal subjects and six patients with PPH, as measured with PET imaging. The mean values are significantly different ($p < 0.05$).

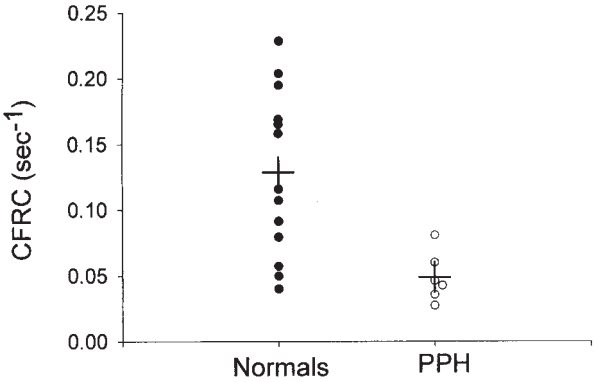

Figure 5. Mean ("+" sign) and individual (*symbols*) values for the CFRC of [^{18}F]FCAP with lung ACE in 13 normal subjects and six patients with PPH, as measured with PET imaging. The mean values are significantly different ($p < 0.05$).

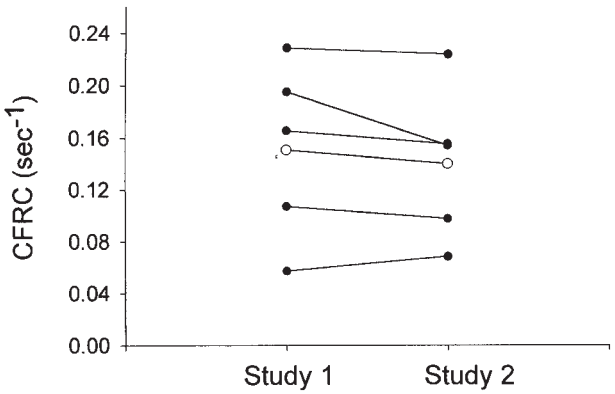

Figure 7. Mean (*empty circles*) and individual (*filled circles*) values for the CFRC of [^{18}F]FCAP with lung ACE in five normal subjects studied on two occasions, as measured with PET imaging. The mean values are not significantly different.

image spatial resolution) by calculating a recovery fraction for the right ventricle activity data as previously described (15).

The time–activity data from multiple ROIs (2 per slice, 6 slices, or 12 per subject) were averaged to yield a single data set per subject. CFRC was then calculated from this set of time–activity data (tissue and right ventricle) as previously reported (14).

Statistical Methods

Data are presented as the mean ± SD. Measurements before and after 1 wk enalapril treatment were compared using a paired Student's *t* test. Baseline values between the normal and PPH groups were compared using an unpaired *t* test. All tests were two-tailed and significance was assigned if $p < 0.05$.

RESULTS

PBF was 109 ± 30 ml/min/100 ml lung for the normal group and 87 ± 12 ml/min/100 ml lung for the PPH group ($p = 0.1$). When PBF values were corrected for body weight, PBF in normals (1.47 ± 0.35 ml/min/kg/100 ml lung) was significantly greater than in PPH patients (1.09 ± 0.33 ml/min/kg/100 ml lung) (Figure 3).

Average Vb was 14.4 ± 2.8 ml blood/100 ml lung (i.e., 14% of the lung volume in the ROIs was occupied by blood) for the normal group and 11.0 ± 3.3 ml/100 ml lung for the PPH group (Figure 4) ($p < 0.05$).

CFRC was 0.128 ± 0.063/s for the normal group and 0.049 ± 0.019/s for the PPH group (Figure 5) ($p < 0.01$). The mean value was approximately 60% lower in PPH patients than the mean value in normal subjects. This relative difference was greater than that seen for either PBF or Vb (Figure 6).

For the five normal volunteers who were scanned twice 1 wk apart without any intervention, CFRC was 0.151 ± 0.067/s for the first measurement and 0.140 ± 0.060/s for the second measurement (Figure 7). The means differed by only 7%, which was not statistically significant ($p = 0.8$). The average change for the five subjects was 12 ± 8% (−21% to +20%). The coefficient of repeatability, defined as the 95% range for the difference in two repeat measurements, was 0.038/s.

In the five normal subjects who were studied before and after enalapril, mean CFRC at the first scan was 0.177 ± 0.053/s. After 1 wk of treatment with enalapril, CFRC decreased (was blocked) in every subject, with a mean value of 0.028 ± 0.017/s ($p < 0.005$ compared with untreated baseline) (Figure 8). The mean percentage reduction was 83% ($n = 5$), with a range of 69% to 99%.

In the five patients with PPH who were studied before and after enalapril, mean CFRC at the first scan was 0.052 ± 0.020/s. After 1 wk of treatment with enalapril, CFRC decreased (was blocked) in every subject (Figure 9). The percentage reduction varied from 62% to 86%. The mean fall (blocking) was 76%.

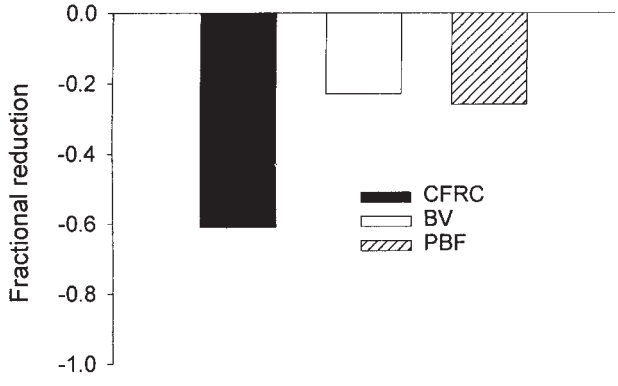

Figure 6. Fractional difference between the mean values of the patients with PPH and normal subjects (i.e., mean PPH value − mean normal value/mean normal value) for the combined forward rate constant of [^{18}F]FCAP with lung ACE (CFRC), regional lung Vb, regional PBF.

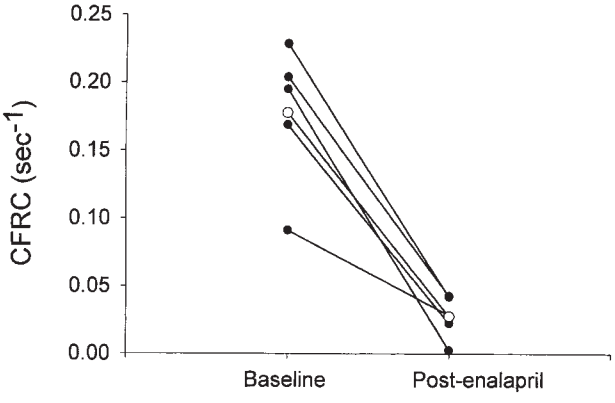

Figure 8. Mean (*empty circles*) and individual (*filled circles*) values for the CFRC of [^{18}F]FCAP with lung ACE in five normal subjects studied before and after 1 wk of treatment with oral enalapril, as measured with PET imaging. The mean values are significantly different.

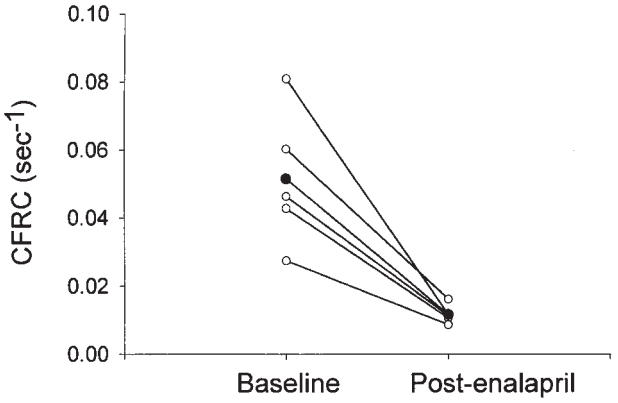

Figure 9. Mean (*filled circles*) and individual (*empty circles*) values for the CFRC of [^{18}F]FCAP with lung ACE in five patients with PPH studied before and after 1 wk of treatment with oral enalapril, as measured with PET imaging. The mean values are significantly different.

Mean CFRC after treatment was 0.012 ± 0.003/s ($p < 0.01$ compared with untreated baseline).

DISCUSSION

The two principal findings of this study are (*1*) the rate of tracer binding for ACE (CFRC) in patients with PPH is substantially lower than in normal subjects, and (*2*) in both patients and normal subjects, a low dose of oral ACE inhibitor effectively blocks more than 75% of lung ACE. These findings suggest that a clinical trial of ACE inhibitors to determine their effect on vascular remodeling and outcome in PPH could be conducted safely.

ACE Binding in Normal Subjects and PPH Patients

Not only is angiotensin II a strong vasoconstrictor and stimulant of aldosterone secretion, as is well known, but it is also a growth factor for vascular smooth muscle and myocardium (19, 20). Because all the components of the renin–angiotensin system required to produce angiotensin II are widely distributed in many tissues (13), ACE inhibitors may help prevent the pathologic proliferation of vascular smooth muscle and myocardium by locally inhibiting angiotensin II production. In addition, because the substrate specificity of ACE is low (it metabolizes bradykinin as well as peptides such as substance P and enkephalin), important local effects other than those produced by angiotensin II per se are also possible.

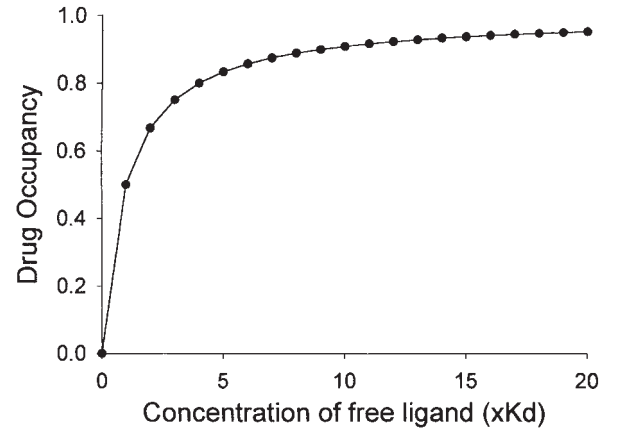

Figure 10. Theoretical relationship between drug occupancy (i.e., B/B_{max}) versus the concentration of free drug [C], expressed in terms of the equilibrium dissociation constant between [^{18}F]FCAP and lung ACE (K_d).

The standard view has been that because pulmonary ACE is present in abundance, it serves no regulatory role in the pathogenesis of hypertensive vascular disease, nor is it rate-limiting in the regulation of angiotensin II. These concepts have been challenged by recent studies. For instance, in cardiac and renal tissue, a variety of experimental models of hypertension are associated with increased local ACE gene transcription, along with appropriately increased levels of ACE activity (21, 22). The administration of ACE inhibitors consistently leads to increased ACE messenger RNA (mRNA) production in experimental animals, especially in the lung (21, 23). Conversely, prolonged administration of angiotensin II leads to reduced levels of ACE mRNA. In cultured bovine pulmonary artery endothelial cells, ACE activity is increased by glucocorticoids, thyroid hormone, and several cyclic-AMP related agents, whereas it is decreased by insulin (24, 25). These various studies strongly suggest that ACE, including pulmonary ACE is indeed regulated, and that while the amount of ACE is always great enough to effectively convert all circulating angiotensin I to angiotensin II, changes in tissue ACE levels may modulate the local production of angiotensin II and bradykinin, with potentially important consequences to tissue morphology and function.

The potential role of tissue ACE in lung vascular remodeling has received relatively little attention, which is surprising given the relatively large amounts of ACE that are present in the lung and its evident importance in systemic vascular disease. Although the effects of a variety of acute injuries (chronic hypoxia, phorbol myristate acetate, radiation, and bleomycin among others) on ACE activity (i.e., either the transpulmonary conversion of circulating angiotensin I to angiotensin II, or the single-pass metabolism of a small peptide substrate for ACE) have been studied frequently (26–31), these studies have usually focused on using ACE activity as a marker of endothelial dysfunction, and not, in general, on whether changes in ACE activity have had any pathophysiologic consequences per se for the development or resolution of the injury.

Clozel and coworkers (32) reported that ACE inhibition completely prevented the development of medial thickening in pulmonary arteries (a cardinal pathologic sign of pulmonary hypertension) in response to chronic hypoxia in rats, suggesting that ACE played an important role in the development of pulmonary hypertension in this model—a finding recently confirmed by Nong and coworkers (33). More recently, Morrell and coworkers reported evidence for increased ACE expression in pulmonary arteries after chronic hypoxia in rats (34).

Several recent studies suggest that ACE may regulate the vascular remodeling found in PPH and experimental models of pulmonary hypertension (9, 11, 35–37). In the current study, we found that the rate of [^{18}F]FCAP binding to ACE in the lung (CFRC) was significantly lower in patients with PPH than in normal subjects. In our model, $CFRC = k_a (B_{max} - B_{occ})$, where B_{max} is the average number of available binding sites under normal conditions, i.e., sites may be occupied by endogenous ligands for ACE (e.g., angiotensin I or endogenous inhibitors) which cannot be measured and are not detectable by our methods. B_{occ} is the number of newly occupied sites as a result of drug treatment; it is assumed that before treatment $B_{occ} = 0$. To interpret CFRC in different groups of patients (normals versus PPH patients in this case), we assume that k_a is similar in the different groups and that $B_{occ} = 0$. To interpret CFRC

in the same group of patients before and after treatment with enalapril, we assume that B_{occ} changes as a result of drug administration, and k_a and B_{max} are unchanged.

Thus, assuming that k_a is unchanged by disease, we interpret the relative decrease in CFRC as indicating a relative decrease in the concentration of ACE binding sites in the lung (B_{max}), which is consistent with results in experimental models of pulmonary hypertension in which total ACE enzymatic activity is reduced compared with normal lungs (37–39). Very recently, Orfanos and coworkers reported that the single-pass transpulmonary hydrolysis of a specific ACE substrate was reduced (consistent with reduced ACE expression) in a heterogeneous group of nine patients with precapillary pulmonary hypertension, including two patients with PPH (40).

On the other hand, we and others have reported that focal ACE gene and protein expression is increased in the neointima of pulmonary arteries of both experimental models and patients with PPH compared with normal pulmonary arteries (9, 10). Thus, a combination of focal increases in ACE expression at sites of vascular remodeling seems to occur simultaneously with an overall decrease in total lung ACE activity or expression (11, 37, 41).

The explanation may lie, partially, in a reduced total endothelial surface area. The endothelial surface of pulmonary arteries is markedly reduced compared with normal pulmonary arteries, because of the smaller internal diameter of the arteries among other reasons. Thus, the total concentration of ACE binding sites in the lungs may be reduced in PPH owing to the dramatically reduced endothelial surface area, even though ACE expression itself is increased locally in remodeling vessels. Indeed, it may well be the case that reductions in ACE binding (as assessed by PET) may correlate with the degree of vascular remodeling.

The overall reduction in the rate of [^{18}F]FCAP binding to lung ACE in this study, however, may not be simply a function of reduced vascular surface area since CFRC was reduced (61% compared with normal subjects) out of proportion to both Vb (23%) and regional pulmonary blood flow (26%). Thus, overall reduced ACE expression, not just reduced activity or vascular surface area, may be a hallmark of pulmonary hypertension (42), even while expression may be increased focally at sites of active remodeling. These relationships among ACE binding (as assessed by PET), vascular surface area, and ACE regulation (focally and in the lung as a whole) will require additional study.

ACE Inhibition as Therapy for PPH

Given evidence for increased ACE expression at sites of vascular remodeling, and given the evidence for the beneficial effects of ACE inhibitors in various clinical conditions associated with systemic vascular remodeling (myocardial infarction, hypertension), it is reasonable to hypothesize that ACE may also be important to the vascular remodeling that accompanies pulmonary vascular disease, especially PPH.

The traditional method to test such a hypothesis in humans would be to initiate a randomized placebo-controlled trial of an appropriate drug, in this case an ACE inhibitor. There are at least two problems with initiating such a trial in PPH. First, the supporting experimental data at present are limited to rodent models of the disease (10, 11, 36, 37, 41). Second, the endpoint for dosing in humans is uncertain. Numerous trials of other vasodilators (with the exception of calcium channel-blockers and prostacyclin) have failed to make a significant impact on the natural history of this disease (2). Interestingly, ACE inhibitors themselves have never been systematically studied as a treatment for pulmonary hypertension. When their use has been reported anecdotally, the focus has always been on their utility and safety in lowering pulmonary artery pressures, not on whether ACE inhibition itself modified the natural history of the disease in any way (43, 44). However, in some cases, clinical improvement did appear to develop, but only after months of treatment (43). Even so, the use of antihypertensives in PPH carries with it the very real risk of clinically important systemic hypotension—possibly, even, resulting in accelerated mortality. Thus, the potential benefit of specifically inhibiting pulmonary ACE, irrespective of any effect on pulmonary artery pressures per se has never been tested. To do so, it would be important to know what dose of drug would be necessary to achieve the goal of specifically inhibiting lung ACE.

In the current study, we have used PET imaging to help determine the dose of the ACE inhibitor, enalapril, that will block uptake of another, in this case radiolabeled, ACE inhibitor, namely [^{18}F]FCAP. Enalapril, the ethylester of enalaprilat, is a prodrug with little pharmacologic activity until hydrolyzed in the liver to enalaprilat. We found that as little as 5 mg orally once a day was sufficient to block, on average, 83% of lung binding to [^{18}F]FCAP in normals and 76% in patients with PPH. Although ACE inhibitors also bind to plasma proteins and to the circulating form of ACE, our model is able to distinguish the radioactivity represented by [^{18}F]FCAP in blood from that due to binding in lung tissue. Thus, the data suggest that a large majority of ACE activity in lung can be inhibited by a low dose of an oral ACE inhibitor.

Whereas the overall lung ACE expression appears to be reduced in patients with PPH compared with normals, the dose required to block lung ACE may still be similar in the two groups. This conclusion is the result of the interactions between ACE and its inhibitors, assuming classical Michaelis-Menten kinetics. Thus, drug occupancy ("inhibition") would be given as follows: $B/B_{max} = [C]/(K_d + [C])$, where B = drug bound to ACE, B_{max} = the maximal binding capacity, [C] is the concentration of free drug, and K_d is the equilibrium dissociation constant (equal to the concentration required to occupy 50% of the available binding sites). Drug occupancy, i.e., B/B_{max}, is therefore related to [C] and K_d, not B_{max}.

The relationship between drug occupancy and free drug concentration is shown in Figure 10. In our study, with average blocking of 76% of ACE binding sites in the PPH patients, the free drug concentration can be estimated to be near the 3 K_d level. Doubling or quadrupling the dose would only produce a marginal additional increase in blocking. Thus, from this analysis, one would conclude that (*1*) the dose of ACE inhibitor required to block lung ACE in patients with PPH and normal subjects will be similar, even though overall lung ACE expression appears to be reduced in patients with PPH and (*2*) larger doses of the drug are unlikely to achieve additional significant lung ACE inhibition.

In conclusion, we report a novel use of PET imaging to estimate organ-specific drug effects. Overall lung ACE expression appears to be reduced in patients with PPH, and only modest doses of an ACE inhibitor appear to inhibit at least 75% of lung ACE. It remains to be determined whether ACE inhibitors, administered over long periods of time, will affect the vascular remodeling that is characteristic of PPH, or whether alternative ACE inhibitors may be more or less effective that the specific agent reported in this study. However, given the low dose of ACE inhibitor that, apparently, is needed to block lung ACE based on the data from the current study, a clinical trial testing the efficacy of ACE inhibition on vascular remodeling and outcome in patients with PPH should be feasible with little additional risk to the patient.

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References

1. Rich, S. 1998. Clinical insights into the pathogenesis of primary pulmonary hypertension. *Chest* 114(Suppl. 3):237S–241S.
2. Rubin, L. J. 1997. Primary pulmonary hypertension. *N. Engl. J. Med*. 336: 111–117.
3. Botney, M. D., and R. P. Mecham. 1994. Cellular mechanism of vascular remodeling in primary pulmonary hypertension. *In* J. Murray and J. Nadel, editors. Textbook of Respiratory Medicine, Vol. 1. W. B. Saunders, Philadelphia. 498–508.
4. Reid, I. 1990. Vascular remodeling. *In* A. P. Fishman, editor. The Pulmonary Circulation: Normal and Abnormal. University of Pennsylvania Press, Philadelphia. 259–282.
5. Giaid, A., M. Yanagisawa, and D. Langleben. 1993. Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. *N. Engl. J. Med*. 328:1732–1739.
6. Bahadori, L., J. Milder, and L. Gold. 1995. Active macrophage-associated TGF colocalizes with type I procollagen gene expression in atherosclerotic human hypertensive pulmonary arteries. *Am. J. Pathol.* 146:1140–1149.
7. Botney, M., L. Bahadori, and L. Gold. 1994. Vascular remodeling in primary pulmonary hypertension: potential role for transforming growth factor. *Am. J. Pathol.* 144:286–295.
8. Liptay, M., W. Parks, R. Mecham, J. Roby, L. Kaiser, J. Cooper, and M. Botney. 1993. Neointimal macrophages co-localize with extracellular matrix gene expression in human atherosclerotic pulmonary arteries. *J. Clin. Invest.* 91:588–594.
9. Schuster, D. P., E. C. Crouch, W. C. Parks, T. Johnson, and M. D. Botney. 1996. Angiotensin converting enzyme expression in primary pulmonary hypertension. *Am. J. Respir. Crit. Care Med.* 154(4, Pt. 1):1087–1091.
10. Tanaka, Y., D. P. Schuster, E. C. Davis, G. A. Patterson, and M. D. Botney. 1996. The role of vascular injury and hemodynamics in rat pulmonary artery remodeling. *J. Clin. Invest.* 98:434–442.
11. Okada, K., M. L. Bernstein, W. Zhang, D. P. Schuster, and M. D. Botney. 1998. Angiotensin-converting enzyme inhibition delays pulmonary vascular neointimal formation. *Am. J. Respir. Crit. Care Med.* 158:939–950.
12. Lee, M. A., M. Bohm, M. Paul, and D. Ganten. 1993. Tissue renin-angiotensin systems: their role in cardiovascular disease. *Circulation* 87:7–13.
13. Dzau, V., and R. Re. 1994. Tissue angiotensin system in cardiovascular medicine: a paradigm shift? *Circulation* 89:493–498.
14. Markham, J., T. J. McCarthy, M. J. Welch, and D. P. Schuster. 1995. In vivo measurements of pulmonary angiotensin-converting enzyme kinetics: I. Theory and error analysis. *J. Appl. Physiol.* 78:1158–1168.
15. Schuster D. P., T. J. McCarthy, M. J. Welch, S. Holmberg, P. Sandiford, and J. Markham. 1995. In vivo measurements of pulmonary angiotensin-converting enzyme kinetics: II. Implementation and application. *J. Appl. Physiol.* 78:1169–1178.
16. Schuster, D. P. 1998. The evaluation of lung function with PET. *Sem. Nucl. Med*. 28:341–351.
17. Hwang, D. R., W. C. Eckelman, C. J. Mathias, E. W. Petrillo, Jr., J. Lloyd, and M. J. Welch. 1991. Positron-labeled angiotensin-converting enzyme (ACE) inhibitor: fluorine-18-fluorocaptopril. Probing the ACE activity in vivo by positron emission tomography. *J. Nucl. Med.* 32:1730–1737.
18. Schuster, D. P. J. D. Kaplan, K. Gauvain, M. J. Welch, and J. Markham. 1995. Measurement of regional pulmonary blood flow with PET. *J. Nucl. Med*. 36:371–377.
19. Schelling, P., H. Fischer, and D. Ganten. 1991. Angiotensin and cell growth: a link to cardiovascular hypertrophy? *J. Hypertension* 9:3–15.
20. Itoh, H., M. Mukoyama, R. Pratt, G. Gibbons, and V. Dzau. 1993. Multiple autocrine growth factors modulate vascular smooth muscle cell growth response to angiotensin II. *J. Clin. Invest.* 91:2268–2274.
21. Schunkert, H., J. Ingelfinger, A. Hirsch, Y. Pinto, W. Remme, H. Jacob, and V. Dzau. 1993. Feedback regulation of angiotensin converting enzyme activity and mRNA levels by angiotensin II. *Circ. Res.* 72:312–318.
22. Shiota, N., M. Miyazaki, and H. Okunishi. 1992. Increase of angiotensin converting enzyme gene expression in the hypertensive aorta. *Hypertension* 20:168–174.
23. King, S., and S. Oparil. 1992. Converting enzyme inhibitors increase converting enzyme mRNA and activity in endothelial cells. *Am. J. Physiol.* 263:C743–C749.
24. Dasarthy, Y., and B. Fanburg. 1991. Involvement of second messenger systems in stimulation of angiotensin converting enzyme of bovine endothelial cells. *J. Cell. Physiol.* 148:327–335.
25. Dasarathy, Y., J. Lanzillo, and B. Fanburg. 1992. Stimulation of bovine pulmonary artery endothelial cell ACE by dexamethasone: involvement of steroid receptors. *Am. J. Physiol.* 263:L645–L649.
26. Hollinger, M., S. Giri, S. Patwell, J. Zuckerman, A. Gorin, and G. Parsons. 1980. Effect of acute lung injury on angiotensin converting enzyme in serum, lung lavage, and effusate. *Am. Rev. Respir. Dis.* 121: 373.
27. Gissis, C., X. Chen, and M. Merker. 1992. Lisinopril and ramiprilat protection of the vascular endothelium against free radical–induced functional injury. *J. Pharmacol. Exp. Ther.* 262:212.
28. Ward, W., P. Lin, P. Wong, R. Behnia, and N. Jalali. 1993. Radiation pneumonitis in rats and its modification by the angiotensin converting enzyme inhibitor captopril evaluated by high resolution computed tomography. *Radiat. Res.* 135:81–87.
29. Riggs, D., A. Havill, B. Pitt, and C. Gillis. 1988. PMA activated neutrophils decrease pulmonary endothelial ectoenzyme activities in perfused rabbit lungs. *Am. J. Physiol.* 263:L650–L656.
30. McCormick, J., R. Chrzanowski, J. Anreani, and J. Catravas. 1987. Early pulmonary endothelial enzyme dysfunction after phorbol ester in conscious rabbits. *J. Appl. Physiol.* 634:1972–1978.
31. Jin, H., S. Oparil, H. S. Ann, R. Yang, and R. M. Jackson. 1987. Hypoxia induced inhibition of converting enzyme activity: role in vascular regulation. *J. Appl. Physiol.* 63:1012–1018.
32. Clozel, J., C. Saunier, D. Harmann, and W. Fischli. 1991. Effects of cilazapril, a novel angiotensin converting enzyme inhibitor on the structure of pulmonary arteries of rats exposed to chronic hypoxia. *J. Cardiovasc. Pharmacol.* 17:36–40.
33. Nong, Z., J. M. Stassen, L. Moons, D. Collen, and S. Janssens. 1996. Inhibition of tissue angiotensin-converting enzyme with quinapril reduces hypoxic pulmonary hypertension and pulmonary vascular remodeling. *Circulation* 94:1941–1947.
34. Morrell, N., E. Atochina, K. Morris, S. Danilov, and K. Stenmark. 1995. Angiotensin converting enzyme expression is increased in small pulmonary arteries of rats with hypoxia induced pulmonary hypertension. *J. Clin. Invest.* 96:1823–1833.
35. van Suylen, R., J. Smits, and M. Daemen. 1998. Pulmonary artery remodeling differs in hypoxia and monocrotaline induced pulmonary hypertension. *Am. J. Respir. Crit. Care Med*. 157:1423–1428.
36. Morrell, N. W., E. N. Atochina, K. G. Morris, S. M. Danilov, and K. R. Stenmark. 1995. Angiotensin converting enzyme expression is increased in small pulmonary arteries of rats with hypoxia-induced pulmonary hypertension [published erratum appears in *J. Clin Invest.* 271]. *J. Clin. Invest.* 96:1823–1833.
37. Morrell, N. W., K. G. Morris, and K. R. Stenmark. 1995. Role of angiotensin-converting enzyme and angiotensin II in development of hypoxic pulmonary hypertension. *Am. J. Physiol.* 169:H1186–H1194.
38. Keane, P. M., J. M. Kay, K. L. Suyama, D. Gauthier, and A. K. Lung. 1982. Angiotensin converting enzyme activity in rats with pulmonary hypertension. *Thorax* 37:198–204.
39. Okada, K., M. L. Bernstein, W. Zhang, D. P. Schuster, and M. D. Botney. 1998. Angiotensin-converting enzyme inhibition delays pulmonary vascular neointimal formation. *Am. J. Respir. Crit. Care Med.* 158:939–950.
40. Orfanos, S., D. Langleben, J. Khoury, R. Schlesinger, L. Dragatakis, C. Roussos, J. Ryan, and J. Catravas. 1999. Pulmonary capillary endothelium bound angiotensin converting enzyme activity in humans. *Circulation* 99:1593–1599.
41. Morrell, N. W., E. N. Atochina, K. G. Morris, S. M. Danilov, and K. R. Stenmark. 1995. Angiotensin converting enzyme expression is increased in small pulmonary arteries of rats with hypoxia-induced pulmonary hypertension. *J. Clin. Invest.* 96:1823–1833.
42. Pfeiffer, M., G. Bruckschlegel, S. Holmer, M. Paul, A. Riegger, and H. Schunkert. 1998. Reciprocal regulation of pulmonary and cardiac angiotensin converting enzyme in rats with severe left ventricular hypertorphy. *Cardiovasc. Res.* 38:125–132.
43. Stumpe, K. O., K. Schmengler, L. Bette, A. Overlack and R. Kolloch. 1986. Persistent hemodynamic and clinical improvement after captopril in patients with pulmonary hypertension. *Herz* 11:217–225.
44. Ikram, H., A. H. Maslowski, M. G. Nicholls, E. A. Espiner, and F. T. Hull. 1982. Haemodynamic and hormonal effects of captopril in primary pulmonary hypertension. *Br. Heart J.* 48:541–545.